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# Therapeutic Strategies for Alzheimer Disease

## *Focus on Neuronal Reactivation of Metabolically Impaired Neurons*

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**Abstract:** Based on several lines of evidence, it has been hypothesized that decreased neuronal metabolic rate may precede cognitive impairment, contributing to neuronal atrophy as well as reduced neuronal function in Alzheimer disease (AD). Additionally, studies have shown that stimulation of neurons through different mechanisms may protect those cells from the deleterious effects of aging and AD, a phenomenon we paraphrased as “use it or lose it.” Therefore, it is attractive to direct the development of therapeutic strategies toward stimulation of metabolic rate/neuronal activity to improve cognition and other symptoms in AD. A number of pharmacological and non-pharmacological approaches discussed here support the concept that stimulation of the brain has beneficial effects and may, to a certain degree, restore several aspects of cognition and other central functions. For instance, the circadian system, which controls the sleep/wake cycle, may be stimulated in AD patients by exposing them to more light or transcutaneous nerve stimulation. We will also discuss a procedure that has been developed to culture human postmortem brain tissue, which allows testing of the efficacy of putative stimulatory compounds.

**Key Words:** circadian system, neuronal metabolic rate, neuronal stimulation

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Alzheimer disease (AD) is a multifactorial disease in which age and the APOE4 allele are important risk factors. The APOE4 genotype is associated with memory decline in cognitively impaired elderly and is present in about 17% of all AD patients.<sup>1–3</sup> In addition, female gender is a risk factor, which agrees with the more severe early cytoskeletal alterations present in the nucleus basalis of Meynert (NBM) of women.<sup>4</sup> Histopathologically, AD is characterized by a large number of

neuritic plaques (NPs) as well as cytoskeletal changes that present as neurofibrillary tangles (NFTs), neuropil threads, or dystrophic neurites.<sup>5</sup> Since these neuropathological changes cannot be distinguished qualitatively from those appearing in elderly nondemented subjects, it is still a controversial issue whether these hallmarks are responsible for the clinical symptoms of dementia or are a byproduct of the disease.<sup>6</sup> During normal aging, cell loss is not a prominent phenomenon. In fact, unaltered neuronal numbers have been reported in many brain areas,<sup>7</sup> and the loss of neocortical neurons over a lifespan was estimated to be only 10%.<sup>8</sup> Regeur et al.,<sup>9</sup> using unbiased sampling and counting methods, showed that despite the generally observed cortical atrophy in AD, global neocortical neuronal loss is not present, providing strong evidence that neuronal shrinkage rather than cell death is a major phenomenon in this neurodegenerative disorder.

Age-related memory disturbances and the loss of memory in AD have been associated, at least partly, with cholinergic dysfunction and degenerative changes in the NBM, which projects to the cerebral cortex.<sup>10</sup> Indeed, neurotoxic lesions of the cholinergic system in experimental animals induce performance deficits,<sup>11</sup> and marked reductions in cholinergic markers have been found in the cerebral cortex even at an early stage of AD.<sup>12</sup> Moreover, the number of neurons containing choline acetyltransferase (ChAT) and the vesicular acetylcholine transporter correlates significantly with the severity of dementia, as determined by the Mini-Mental State Examination (MMSE).<sup>13</sup> Despite these and related observations, neuronal loss in the NBM appears less extensive than presumed earlier. Here we focus on the NBM to examine the evidence that decreased neuronal activity via neuronal atrophy plays a role in AD pathology, including a discussion of the possible contribution of reduced cellular metabolism and reduced neurotrophin activity to this pathologic process. Additionally, we consider the therapeutic possibilities of pharmacological and nonpharmacological approaches to neuronal reactivation.

### NEURONAL ATROPHY RATHER THAN LOSS IN THE NBM

Estimations of neuronal loss in the NBM during normal aging vary greatly, with decreases ranging from 23% to

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90%<sup>14–20</sup> to no significant neuronal loss.<sup>21,22</sup> Massive cell death in the NBM was originally presumed to be one of the major hallmarks of AD,<sup>14,16,18,23,24</sup> and a clear loss of ChAT, a marker of NBM neurons, was reported.<sup>25</sup> However, it is of crucial importance to distinguish loss of a cholinergic marker from a loss of neurons. It has been presumed that the large differences in cell loss that were reported may partly be due to heterogeneity of different NBM subdivisions.<sup>26</sup> Indeed, Vogels et al.<sup>27</sup> found an overall neuron loss in the NBM of only 10% in AD patients, while neuron loss varied from 0% in the rostral to 36% in the caudal part of the NBM. However, regional heterogeneity cannot be the only explanation for the variable data reported. Even studies performed on one particular NBM subdivision showed considerable variation. For instance, measurements performed in the NBM sub-area Ch4a showed cell loss differences varying between 42% and 89%<sup>16,20</sup> to no significant cell loss.<sup>25</sup>

The most likely explanation for the equivocal results concerning neuronal loss in the NBM in AD is the use of different criteria for the size of counted cells. Mann et al.,<sup>16</sup> for instance, only counted cells meeting a minimum diameter requirement and reported a 54% cell loss in the NBM, whereas Pearson et al.<sup>25</sup> counted all NBM neurons regardless of their size and did not find any significant cell loss in the NBM. Indeed, while the number of large neurons decreases, the number of small neurons increases in the NBM in AD.<sup>15,27–29</sup> The combined data indicate that the majority of large neurons atrophy and lose their cholinergic markers but only a small subset dies.

### METABOLIC ACTIVITY IN THE NBM IN RELATION TO APOE GENOTYPE

Neuronal atrophy is an indicator of decreased cellular metabolism, which has been extensively studied as a contributing factor to AD. The size of the Golgi apparatus (GA), part of the protein processing and targeting machinery, has been used as a sensitive parameter for measuring changes in neuronal metabolic activity.<sup>30,31</sup> GA size was, therefore, used in our studies to monitor metabolic activity changes in the NBM in aging and AD.

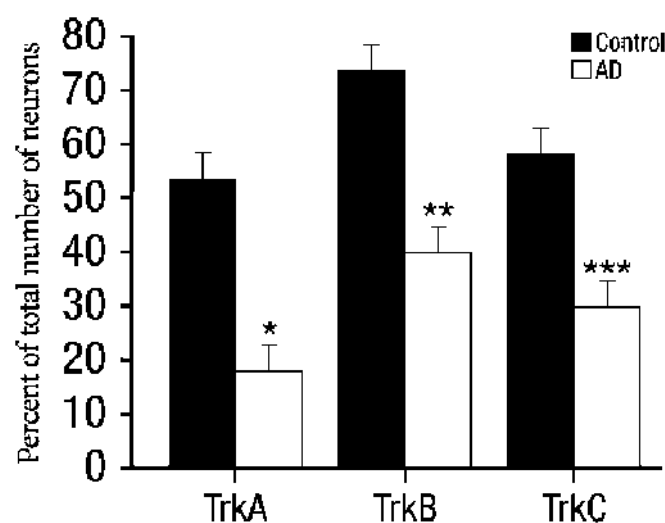
A strong decrease in GA size (49%) was observed in AD, suggesting that the capacity of NBM neurons to process and target proteins decreases dramatically in the disease.<sup>32,33</sup> This notion is consistent with previous demonstrations of decreased nucleolus volume<sup>16,34</sup> and reduced ChAT and cholinesterase activity in the NBM in AD.<sup>17,18,35–37</sup> This reduction in metabolic rate is amplified in those AD patients with either one or two APOE4 alleles,<sup>33</sup> and this finding is in full agreement with the more severe cholinergic deficit in the temporal cortex observed in AD patients with one or two APOE4 alleles.<sup>38</sup>

Postmortem temporal cortex tissue obtained from cognitively normal subjects with an APOE4 allele showed compromised cholinergic activity compared with tissue from sub-

jects without this allele.<sup>39</sup> Therefore, we currently are investigating whether neuronal metabolism is already lower in the NBM of APOE4-positive, cognitively normal subjects without AD pathology (i.e., Braak stage 0–II), compared with APOE4 negative controls. Using the size of the GA as a measure of neuronal metabolism, this indeed appears to be the case (E.J.G. Dubelaar et al., unpublished observation), lending support to a role for decreased metabolism as a risk factor in AD.

### NEUROTROPHIN RECEPTOR CHANGES IN THE NBM

Neurotrophins, including nerve growth factor (NGF), promote neuronal metabolism and function, suggesting that disruption of neurotrophin signaling could play a role in the neuronal atrophy seen in AD. In the basal forebrain complex, which includes the NBM, both low affinity neurotrophin receptors (*p75*)<sup>40,41</sup> and high affinity neurotrophin receptors<sup>42</sup> are present. All three family members of the high affinity receptors, the tyrosine receptor kinases (trks) A, B, and C, are found in NBM neurons.<sup>43–45</sup> In the NBM, levels of NGF and NGF receptors decrease during aging and even more so in AD.<sup>46,47</sup> Studies from our group show that all three types of trks colocalize in NBM neurons and are decreased at varying levels in AD (trkA levels < trkB < trkC)<sup>45</sup> (Fig. 1). TrkA mRNA levels decrease markedly in AD,<sup>48</sup> and reduction in trkA expression has subsequently been confirmed.<sup>49,50</sup> More-



**FIGURE 1.** Graph depicting the proportion of neurons stained by trk antibodies in AD patients and controls in the nucleus basalis of Meynert (NBM). Note the strong reduction in the proportion of trkA-expressing neurons in Alzheimer patients, which is followed by trkB and trkC (\* $P = 0.00001$ , \*\* $P = 0.009$ , \*\*\* $P = 0.004$ ). (Data from Salehi et al.<sup>45</sup>) Reprinted from *Neuroscience*, V75, Salchi A, Verhaagen J, Dijkhuizen PA, et al., "Co-localization of high-affinity neurotrophin receptors in nucleus basalis of Meynert neurons and their differential reduction in Alzheimer's disease." 373–387, 1996, with permission from Elsevier.

over, it was shown that a loss of immunoreactive trkA neurons occurs in individuals with mild cognitive impairment (MCI) without dementia, to the same degree as in early AD.<sup>51</sup> The reduction in trk receptors may underlie the diminished NGF levels in the NBM (due to a decrease in NGF retrograde transport), leading to decreased metabolism and function of NBM neurons. Indeed, a defect in retrograde transport of NGF in the NBM of AD patients has been observed.<sup>47,52</sup>

In addition to changes in levels of the high affinity trk receptors, we have found that the low affinity pan-neurotrophin receptor, *p75*, showed significantly decreased levels both in cell bodies and in fibers of the NBM of AD patients.<sup>53</sup> It thus appears that both high and low affinity neurotrophin receptors are decreased in the NBM of AD patients, with reductions in both receptor types possibly contributing to the defect in NGF retrograde transport. Yet, this defect also may be related to cytoskeletal changes in the NBM that are presumed to hamper axonal transport in AD.<sup>54</sup>

The precise manner in which decreased neuronal metabolic activity,<sup>32</sup> cytoskeletal changes,<sup>54</sup> loss of trk and *p75* receptors,<sup>45,53</sup> and failed NGF retrograde transport<sup>55</sup> are related to the neuronal atrophy and diminished function of the NBM neurons should be studied further. While a clear role for NGF therapy in AD remains to be elucidated, a few AD patients treated with low doses of NGF infused into the cerebrospinal fluid experienced several serious side effects, including pain and weight loss. The pain dissipated shortly after NGF infusion was halted and was followed by weight gain.<sup>56,57</sup> Another approach that currently is being studied in AD patients is NGF gene therapy, which almost completely reverses the loss of NBM neurons in rhesus monkeys.<sup>58</sup>

### DECREASED NEURONAL ACTIVITY IS A WIDESPREAD PHENOMENON IN AD

Various observations in addition to those mentioned above for the NBM indicate that decreased neuronal activity is a widespread, essential characteristic of AD, either as a risk factor or as a direct pathogenic factor,<sup>59</sup> while a high or enhanced neuronal activity would protect against the degenerative changes of aging or AD, a hypothesis we paraphrased as “use it or lose it.”<sup>60</sup> Although a comprehensive review of the data is beyond the scope of this article (for review see Swaab et al.<sup>6</sup>), we will briefly discuss some of the more salient findings. The report that the postmortem AD brain shows a lower total amount of protein,<sup>61</sup> a clear reduction in total cytoplasmic RNA<sup>62–64</sup> and messenger RNA,<sup>65–67</sup> a smaller cell size (e.g., the somatostatin neurons in the cortex),<sup>68</sup> and a small size of the neuronal GA<sup>32,69,70</sup> are all indications of neuronal atrophy and decreased metabolic activity in AD. These findings agree with the observed reduction in glucose metabolism found in the AD brain, particularly in the temporal and parietal lobes, as shown by positron emission tomography.<sup>71–74</sup> Moreover, decreases in regional cerebral glucose metabolism as measured

by positron emission tomography in the temporoparietal, prefrontal, and occipital cortices have been correlated with a change in MMSE score in probable AD patients, providing further evidence that clinical deterioration and metabolic impairment are closely related.<sup>75</sup>

### METABOLIC RATE UNCHANGED BY NEUROFIBRILLARY TANGLES AND NEURITIC PLAQUES

Since the finding of decreased metabolic rate in affected brain areas in AD, the two major questions concerning the pathogenesis of AD have been (1) whether the presence of NPs or NFTs in AD is related to decreased neuronal activity in various brain areas and, if so, (2) whether these neuropathological hallmarks induce decreased metabolic rate or vice versa. Alternatively, neuropathological changes and a decreased metabolic rate could occur independently. Our research supports the latter possibility.

To study the causality of the relationship between NPs and NFTs and decreased metabolic activity, we compared metabolic activity (by measuring GA size) of CA1 neurons that contained NFTs to those that lacked this neuropathological feature. Since we found no apparent difference in the size of the GA between these two groups of neurons, the presence of NFTs does not seem to cause an extra decrease in the general metabolic rate of a neuron.<sup>69</sup> So, although NFTs and decreased metabolic activity are present in the same brain area (i.e., CA1), they do not appear to be causally related. This observation is in agreement with Gertz et al.,<sup>76</sup> who showed that the presence of intraneuronal NFTs in the CA1 area of the hippocampus is not related to another parameter of general metabolic activity (i.e., nucleolar or cell size). These observations, however, certainly do not exclude the possibility that NFTs may decrease the production of specific compounds. Indeed, Häntänpää et al.<sup>77</sup> reported that cytochrome oxidase subunit III mRNA is decreased in NFT-bearing neurons.

NPs are considered by some investigators as later stages of amorphous plaques.<sup>78</sup> Because of extensive damage to the neuropil in the vicinity of NPs, they are also called “malignant” plaques.<sup>79</sup> Although it is still a matter of controversy, many investigators believe that the  $\beta$ -amyloid content of the core plaque is neurotoxic and induces neural degeneration. However, unlike the case of NFTs, there is no clear relationship between the number of NPs and the severity of dementia, which may call into question whether the neurotoxic effects of NPs are a major pathogenetic mechanism in AD. If NPs indeed contain neurotoxic compounds, one would expect that the closer a neuron is situated to an NP, the lower its metabolic rate. Our experiments do not support such a mechanism, as there appears to be no relationship between the metabolic activity of neighboring neurons and either the density of NPs or the distance to each NP.<sup>33</sup> Therefore, our findings do not support the notion that neurotoxicity of NPs causes decreased neu-



ronal metabolism but rather suggest decreased neuronal metabolism and NP neurotoxicity are independent phenomena.

### REACTIVATION AS A MEANS OF RESTORING NEURONAL FUNCTION IN AD

As this review has discussed, there appears to be reduced neuronal metabolic activity in various brain areas in AD patients. Consequently, one may assume that restoration of neuronal activity, either by pharmacological or nonpharmacological approaches, would decrease cognitive impairment.<sup>6,60</sup> Although it is not yet clear whether decreased metabolic activity is a primary process in the pathogenesis of AD, recent data show that reactivation of neurons is possible in the elderly and, in principle, beneficial to AD patients.

#### Pharmacological Approaches to Neuronal Reactivation

One of the neurotransmitter systems clearly affected in AD is the cholinergic system. Cholinesterase inhibitors (ChEIs) enhance acetylcholine content in the synaptic cleft and are associated with positive effects on cognitive function.<sup>10</sup> In some cases, the partial restoration of acetylcholine receptor functioning via ChEIs has paralleled increased cerebral blood flow and glucose metabolism.<sup>80</sup> The beneficial cognitive effects of ChEIs may involve increased neuronal metabolism and, therefore, may increase neuronal activity. An agent in another drug class, Neotrofin, is a reported inducer of neurotrophins and may confer memory improvement in AD patients via the induction of metabolic changes in various brain areas.<sup>81</sup> Hormone therapy also is under investigation, as estrogen was shown to significantly increase glucose metabolism in the lateral temporal region of nondemented elderly people.<sup>82</sup> Epidemiological evidence indicated that estrogen replacement therapy in postmenopausal women was associated with a lower risk and/or delayed onset of AD<sup>83</sup>; however, randomized controlled trials in AD patients have shown mixed results.<sup>83</sup>

#### Nonpharmacological Approaches to Neuronal Reactivation

Engagement in both intellectual and leisure activities and/or the participation in cognitively stimulating activities may reduce the risk of AD.<sup>84–86</sup> The mechanism behind the concept of increased brain reserve through environmental stimulation, also termed by us as “use it or lose it,”<sup>60</sup> is not well understood; however, important contributions have been made, including those of Snowdon and colleagues through their study of aging and disease in religious clergy (the “Nun Study”).<sup>87</sup> In one analysis, the idea density in subjects’ autobiographies was found to predict within about 85% to 90% accuracy which individuals would present with AD nearly 60 years later (low idea density was associated with AD). One interpretation of this finding is that early cognitively stimulating environmental factors may well lead to better brain function and reserve, lowering risk for AD.

To directly test the effects of neuronal stimulation, various types of peripheral nerve stimulation (i.e., transcutaneous electrical nerve stimulation [TENS], tactile nerve stimulation, and a combination of both types of stimuli) were used to assess the effects of increased somatosensory input on memory, and on independent and affective functioning of patients in relatively early stages of AD.<sup>88–92</sup> These studies show that, compared to controls who received a placebo treatment, various aspects of nonverbal short-term memory, nonverbal and verbal long-term memory, and word fluency of stimulated AD patients improved. More specifically, these improvements imply that after stimulation patients were better capable of learning new material, retrieving familiar, categorized information from their memory store, and storing, reversing, and reproducing nonverbal information. With respect to independent and affective functioning, stimulated patients participated more independently in daily life, showed a better personal orientation and orientation in place, and enhanced their social interaction with fellow residents. In addition, stimulated patients felt less withdrawn, irritable, moody, dejected, and gloomy and appeared to be more active and alert, possibly resulting in decreased forgetfulness. In these studies, the therapist was present during both the peripheral stimulation of the experimental group and the placebo treatment of the control group; therefore, an effect of interpersonal communication could not be excluded. Consequently, it was examined whether TENS, in the absence of a therapist, could also have positive effects on the cognitive, independent, and affective functioning of AD patients.<sup>92</sup> The results show that the improvements in nonverbal short- and long-term memory, verbal long-term memory, and word fluency are due solely to the electrical stimulus itself. Furthermore, stimulated patients participated more independently in activities of daily life; however, TENS performed in the absence of a therapist did not appear to have a beneficial effect on patients’ affective functioning. Another finding was that the circadian rest-activity rhythm of stimulated AD patients improved, implying an increase in the strength of coupling to Zeitgebers (i.e., environmental cues).<sup>93</sup> Of note, the majority of the effects of peripheral nerve stimulation, both in the presence and absence of the therapist, could not be maintained during a treatment-free period of 6 weeks.

In the above-mentioned studies, TENS was applied to patients in a relatively early stage of AD; therefore, the question arose as to whether TENS would exert similar positive effects in a more advanced stage of dementia. Consequently, TENS was applied to AD patients (Global Deterioration Scale stage 6, mean MMSE score 4.4 [range 3–7]),<sup>94</sup> and the results showed that only patients’ visual working memory significantly improved; no effects were observed on other aspects of memory processes or affective behavior. It is noteworthy, though, that TENS appeared to have a beneficial effect on patients’ circadian rest-activity rhythm, similar to the observations in patients with an early stage of AD.<sup>95</sup>

As previously emphasized, the “use it or lose it” concept is not only applicable to AD but also to aging.<sup>60</sup> The results of a recent study show that TENS improved visual short-term and verbal long-term (recognition) memory, and semantic verbal fluency in nondemented elderly people.<sup>96</sup> Moreover, stimulated elderly subjects felt less depressed. Of note, the majority of the observed effects could not be maintained during a treatment-free period of 6 weeks, irrespective of dementia stage and presence of a therapist.

A type of stimulation related to TENS is transcranial electrostimulation (TCES, or TES). In one study, TCES in elderly patients with multi-infarct dementia was found to decrease wandering and nocturnal delirium and to enhance patients’ interaction with others.<sup>97</sup> The authors suggest that this effect might be partly mediated through the somatosensory system. TCES recently has been applied to patients in an early stage of AD. The results, however, showed no improvement in cognition or affective behavior.<sup>98</sup> Further studies are required before firm conclusions on the effectiveness of TCES in AD can be drawn.

In addition to nonpharmacological approaches targeting cognitive function, other AD symptoms may respond well to nonpharmacological intervention. An age-related decrease in circadian modulation has been observed for several parameters, including hormone levels, temperature, electroencephalographic activity, alertness, and sleep.<sup>99–101</sup> Elderly people start napping during the day and often complain of disturbed sleep during the night (reviewed by Van Someren<sup>101</sup>), and in AD, this fragmentation of the sleep/wake pattern is even more pronounced. Such disturbances place strong demands on AD caregivers and are among the most important reasons for institutionalization.<sup>100</sup> The suprachiasmatic nucleus (SCN), which is the biologic clock of the brain, is of critical importance in the circadian modulation of behavior and physiology. Combined anatomic, physiological, and behavioral findings suggest that a dysfunctional clock may underlie the sleep/wake pattern fragmentation in the elderly and in AD patients.<sup>102–104</sup> Therefore, we tried a number of strategies designed to stimulate the circadian timing system to enhance the functionality of the clock. Stimulation of the circadian timing system can be effected by means of bright environmental light, peripheral nerve stimulation, and increased levels of physical activity. Our studies in aged rats have demonstrated improvement of both functional and anatomic signs of the circadian timing system after environmental stimulation. Moreover, Witting et al.<sup>105</sup> demonstrated that the decreased amplitude in the circadian distribution of sleep and wakefulness in old rats could be restored to the level of young rats by increasing the intensity of daytime environmental light. In humans, we have used the rest-activity rhythm as a marker for the functionality of the circadian timing system because this variable can be easily assessed using an actigraph, which is a small wrist-worn solid-state recorder that continuously assesses activity level, resulting in a time series

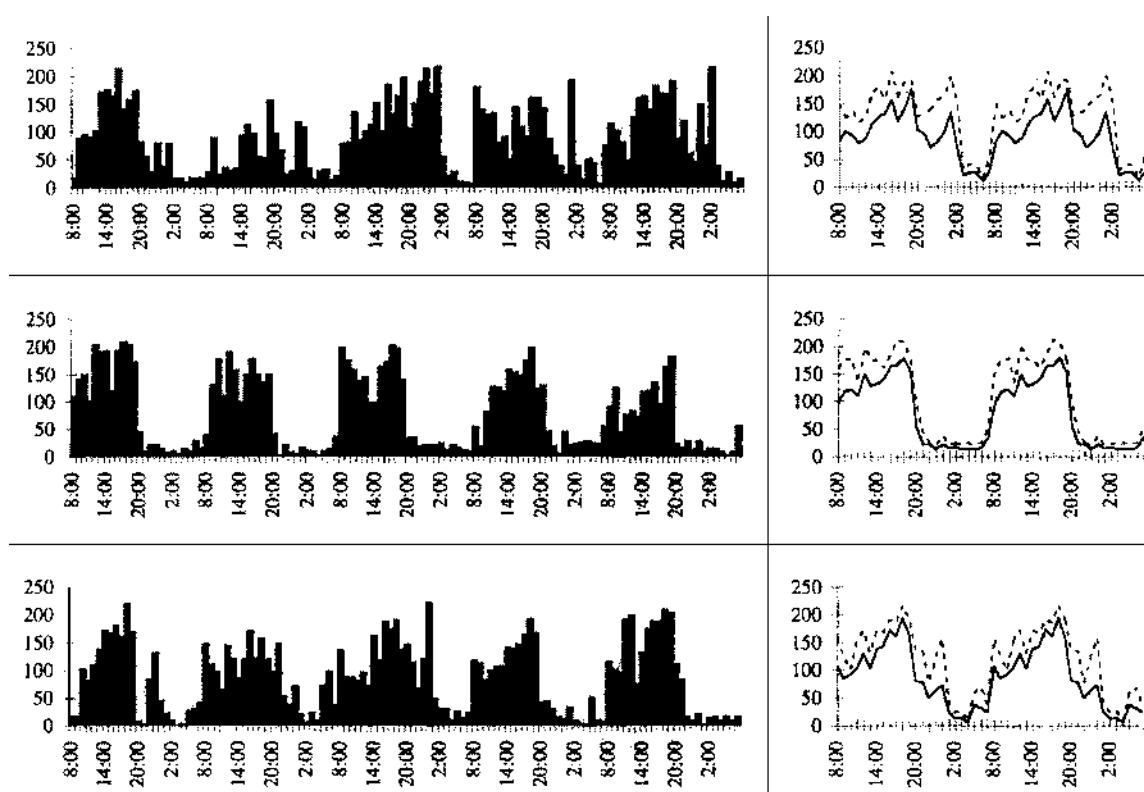
from which the strength of the circadian rhythm can be calculated. In a correlational study, we investigated which constitutional and environmental factors were related to the severity of rhythm disturbances in AD patients. Regression analyses showed the most severe rest-activity rhythm disturbances in patients with a sedentary lifestyle and in patients exposed to low levels of environmental light.<sup>106</sup> Subsequently, we investigated the effect of additional bright light on rest-activity rhythm disturbances in demented patients. Additional bright light improved the coupling of rest-activity rhythms to stable environmental cues (so-called Zeitgebers) in patients with intact vision (Fig. 2), but not in patients with severely compromised sight (partial blindness, cataract).<sup>107</sup> These results agree with other studies showing improved circadian rhythms and decreased behavioral disorders in AD patients treated with bright light.<sup>108–111</sup> The observation that light therapy also increases MMSE scores in demented patients (pretreatment mean MMSE score  $15.2 \pm 4.8$ ; posttreatment mean MMSE score  $18.1 \pm 4.5$ ) makes light therapy of even greater interest for AD research.<sup>112</sup>

Whereas the effect of light on the circadian timing system is well documented, the possible effect of somatosensory input to the SCN has only relatively recently been suggested by our group.<sup>93</sup> In rats and squirrel monkeys, it has been demonstrated that the SCN is innervated by direct spinohypothalamic projections conveying somatosensory information.<sup>113,114</sup> We therefore investigated whether additional somatosensory input by means of TENS would provide an alternative means for the activation of SCN neurons. Indeed, in early-stage demented elderly people, repeated TENS was found to improve the coupling of rest-activity rhythms to Zeitgebers, whereas placebo treatment was ineffective.<sup>93</sup> Similar effects also could be obtained in later stages of AD.<sup>95</sup> The anatomic and functional findings from the reported studies in animals and humans indicate that the SCN retains considerable plasticity during aging and in demented elderly subjects.

## POSTMORTEM PLASTICITY

Animal models for human neurologic and psychiatric diseases only partially mimic the underlying pathogenic processes. Therefore, we explored the potential use of postmortem brain tissue cultures from adult neurologic patients and controls.<sup>115–117</sup> Organotypic human brain cultures obtained by autopsy within the framework of the Netherlands Brain Bank at 2 to 8 hours after death were maintained *in vitro* for extended periods and manipulated experimentally. Slices in basal medium supplemented with survival-promoting neurotrophic factors retain more viable cells than slices in basal medium alone. Mitochondrial activity as measured by cytochrome oxidase activity could be enhanced by the addition of pyruvate as an extra energy source to the medium.

We have found that neurons in these cultures (motor cortex, hippocampus, and cerebellum of adult postmortem brain)



**FIGURE 2.** Raw activity data (left panels) of a patient with AD assessed three times for 5 days: before (upper left panel), during (middle left panel), and after (lower left panel) light treatment. The right panels show double plots of the average 2- to 4-hour activity level (solid line) and one standard deviation above this level (dashed line). Note the decreased variability, the smoother average, and the clearer difference between the day and the night during light treatment. (Data from Van Someren et al.<sup>107</sup>) Reprinted from *Biological Psychiatry*, V41, Van Someren EJW, Kessler A, Mirmiran M, et al, "Indirect bright light improves circadian rest-activity rhythm disturbances in demented patients." 955–963, 1997, with permission from Society of Biological Psychiatry.

can be transduced with adeno-associated viral vectors expressing the reporter genes eGFP (enhanced green fluorescent protein) and lacZ for as long as 44 days.<sup>115–117</sup> These slice cultures offer new opportunities to study the cellular and molecular mechanisms of aging and neurodegenerative diseases such as AD, and to investigate potential treatments, including neuronal reactivation.

## CONCLUSION

An increasing body of evidence indicates that metabolic impairment may contribute to neuronal dysfunction and atrophy in AD. The observations that glucose administration or increasing glucose availability by experimentally inducing hyperinsulinemia enhances memory in patients with probable AD<sup>118–120</sup> support the view that AD is at least partially a hypometabolic disease and also indicate that metabolic stimulation of neurons appears to be a promising strategy.

Stimulation of neurons by other means, both pharmacological and nonpharmacological, also may at least partly reverse degenerative changes in aging and AD. The beneficial effects of several types of neuronal stimulation may differ between age groups, stages of AD, APOE genotype, and status of

functional brain reserves, whether or not induced by early exposure to a complex environment, profession, or education. All these factors may impact the effectiveness of these strategies. Furthermore, effectiveness may strongly depend on the use of appropriate stimuli and the presence of receptors for certain stimulating factors. Finally, human neurons in tissue culture may serve as a model to test many stimulatory approaches, with the ultimate goal of increasing neuronal maintenance during aging and in AD.

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